bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Protein interaction analysis in Y2H system

Vladimir Denic HY Houqing YU

Updated date: Sep 1, 2022

An abbreviated version of this protocol was published in eLIFE in Apr 2022

The peroxisomal exportomer directly inhibits phosphoactivation of the pexophagy receptor Atg36 to suppress pexophagy in yeast

DOI: 10.7554/eLife.74531

Detailed protocol

Protein interaction analysis in yeast-two-hybrid (Y2H) system

Used in Yu *et al*., *Elife*. 2022 Apr 11;11:e74531.

1. Transform the Y2H plasmids (pGADT7 and pGBKT7 derived plasmids) into *S. cerevisiae* strains Y2H gold (Clontech Laboratories) with lithium acetate/polyethylene glycol method developed by Gietz and Woods (*Methods Enzymol*. 2002; 350:87-96).
2. Inoculate single colonies from yeast transformation plates into 3 ml SD -Leu, -Trp medium, grow the cells on culture rotator at 30 ^{O}C overnight
3. Inoculate the overnight culture into fresh 3 ml SD -Leu, -Trp medium (OD_{600}=0.1), grow the cells on culture rotator at 30 ^{O}C for 6 hrs to OD_{600} ~0.8
4. Pellet the cells with centrifugation at 3000 × g for 5 min
5. Wash the cells twice with autoclaved water
6. Resuspend the cells in 1 ml autoclaved water, measure the OD_{600} and dilute the cells to OD_{600}=0.1
7. Place 10 uL dilution of cells in test (OD_{600}=0.1) as one droplet on the selective plates (SD -Leu, -Trp or SD -Leu -Trp, -His, with 10 mM 3-amino-1,2,4-triazole [3-AT])
8. Air-dry the droplets on the plates and seal the plates with parafilm
9. Grow the cells at 30 oC for 2-3 days
10. Take picture of the colonies on the plates to analyze the protein interaction under test

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Denic, V. and YU, H. (2022). Protein interaction analysis in Y2H system. Bio-protocol Preprint. bio-protocol.org/prep1896.
2. Yu, H., Kamber, R. A. and Denic, V.(2022). The peroxisomal exportomer directly inhibits phosphoactivation of the pexophagy receptor Atg36 to suppress pexophagy in yeast. eLIFE. DOI: 10.7554/eLife.74531